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Pyrosequencing and phenotypic microarray to decipher bacterial community variation in *Sorghum bicolor* (L.) Moench rhizosphere

Ashwani Kumar [a,b,1,*], Anamika Dubey [b], Muneer Ahmad Malla [c], Joanna Dames [a]

[a] Mycorrhizal Research Laboratory, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa
[b] Metagenomics and Secretomics Research laboratory, Department of Botany, Dr. Harisingh Gour University (Central University), Sagar 470003, MP, India
[c] Department of Zoology, Dr. Harisingh Gour University (Central University), Sagar 470003, MP, India

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ABSTRACT

Different cultivation practices and climatic conditions play an important role in governing and modulating soil microbial communities as well as soil health. This study investigated, for the first time, keystone microbial taxa inhabiting the rhizosphere of sweet sorghum (*Sorghum bicolor*) under extensive cultivation practices at three different field sites of South Africa (North West-South (ASHSOIL1); Mpumalanga-West – (ASHSOIL2); and Free State-North West – (ASHSOIL3)). Soil analysis of these sites revealed differences in P, K, Mg, and pH. 16S rRNA amplicon sequencing data revealed that the rhizosphere bacterial microbiome differed significantly both in the structure and composition across the samples. The sequencing data revealed that at the phylum level, the dominant group was Cyanobacteria with a relative abundance of 63.3%, 71.8%, and 81.6% from ASHSOIL1, ASHSOIL2, and ASHSOIL3, respectively. Putative metabolic requirements analyzed by METAGENassist software revealed the ASHSOIL1 sample as the prominent ammonia degrader (21.1%), followed by ASHSOIL3 (17.3%) and ASHSOIL2 (11.1%). The majority of core-microbiome taxa were found to be from Cyanobacteria, Bacteroidetes, and Proteobacteria. Functionally, community-level physiological profiling (CLPP) analysis revealed that the metabolic activity of the bacterial community in ASHSOIL3 was the highest, followed by ASHSOIL1 and ASHSOIL2. This study showed that soil pH and nutrient availability and cultivation practices played significant roles in governing the bacterial community composition in the sorghum rhizosphere across the different sites.

1. Introduction

Sorghum (*Sorghum bicolor*) (L.) Moench) is one of the major crop of Africa (Mareque et al., 2018; Dubey et al., 2019a) and it is globally cultivated as a multipurpose crop, grown with low input, serving as food and animal feed, as well as a biofuel feedstock (Ratnavathi et al., 2011). This plant is adapted to elevated temperatures and easily adjusted to extreme conditions of salinity and drought (Dubey et al., 2019a). In South Africa, the Free State Province is the largest sorghum producing area, with more than half (52%) of the total production followed by Mpumalanga (24%), > Limpopo (15%), North West Province (7%), and Gauteng (2%) (Zwane, 2019).

Different cultivation practices and climatic conditions play an important role in governing and modulating soil microbial communities in particular and soil health in general (Dubey et al., 2019b). There are a lot of published articles available supporting the role of these microbial communities in plant growth promotion and biocontrol of various diseases (Hashem et al., 2017). Though, how these microbial communities around and inside the plants are shaped is still unexplored. Under stress conditions, a plant's root releases some exudates to cope with the stress, but on the other hand, it attracts the microbes of their choice by specific binding towards the roots and associated with the rhizosphere or endosphere (Dubey et al., 2020a). Therefore, investigation of the taxonomic and functional composition of the microbial community associated with plants and soil is very urgent to deal with future food crisis and climate change impact on crop productivity limitation (Vyas et al., 2019; Dubey et al., 2021). This indicated that understanding and exploring the ecology of the rhizosphere microbiome is a key to enhancing plant productivity and ecosystem functioning (Dubey et al., 2019b). Apart from the soil types, other factors such as plant species, developmental stage, and climatic conditions are the major determinants of the composition of rhizosphere microbial communities (Mathur et al., 2017). Soil microbiome research has significant potential for helping to understand the effects of soil microbial taxa on the health and productivity of plants and for shaping key ecosystem processes (Dubey et al., 2020a; Kumar and Dubey, 2020). Plant roots

* Corresponding author at: Metagenomics and Secretomics Research laboratory, Department of Botany, Dr. Harisingh Gour University (Central University), Sagar 470003, MP, India.
E-mail address: ashwaniiitd@hotmail.com (A. Kumar).
[1] Claude Leon Foundation Fellow.

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grow deep into the soil and are continuously in contact with soil microbes. The rhizosphere is the area immediately surrounding the plant roots and is a critical zone of the soil in which diverse microbial communities are involved in many key processes required for ecosystem functioning, including the exchange of nutrients between plants, microorganisms, and their associated soil environment (Mendes et al., 2013; Kaushal et al., 2020). The rhizosphere microbiome contains plant root exudates, is determined by plant genotype and type of soil (Kumar et al., 2015), and may contain up to 10^{11} microbial cells per gram of root (Egamberdieva and Lugtenberg, 2014; Azarbad et al., 2018). Microbes help to increase the bioavailability of important mineral nutrients such as N, P, and K (Wallenstein, 2017) and also suppress soil-borne diseases (Nihorimbere et al., 2012; Hashem et al., 2017). Knowledge of the taxonomic composition, and the functional role of these microbial taxa, is essential for sustainable agricultural practice and for reducing dependence on chemical pesticides and fertilizers (Hartmann et al., 2015; Liao et al., 2018).

Soil characteristics are important factors that affect soil microbial diversity (Faoro et al., 2010; Wang et al., 2017). These soil microbes directly influence the structure and composition of the aboveground plant community by promoting plant growth, enhancing stress tolerance, and mediating local patterns of nutrient cycling (Dubey et al., 2020b). Though, soil pH is also an important determinant of soil health. Lauber et al. (2009) reported on the role of soil pH on the soil microbiota. The microbial community structure, diversity, and functioning in the soil are essential determinants of plant health and productivity and these interactions are difficult to study using cultivation-dependent approaches. However, next-generation sequencing (NGS), has recently emerged as a tool for DNA analysis to explore microbial diversity, from the sample collected directly from the environment (Malla et al., 2019, 2018). The development of next-generation sequencing and bioinformatics tools has led to a surge of research endeavors that have rapidly advanced our understanding of the composition and functioning of bacterial populations in very diverse environments (Bolhuis et al., 2014; Huttenhower et al., 2014; Norman et al., 2014; Yoon et al., 2015; Jovel et al., 2016). Several studies have investigated the effect of different agronomical practices on soil microbial communities (Hashem et al., 2018; Ahmad et al., 2018; Bagri et al., 2018; Kumar et al., 2016). This approach has been utilized previously to investigate the composition of microbial communities associated with different plants, such as rice (Edwards et al., 2015), maize (Benitez et al., 2017; Peiffer et al., 2013) *Arabidopsis* (Bulgarelli et al., 2012), and *Populus deltoides* (Gottel et al., 2011; Shakya et al., 2013). However, very few reports have been available on the bacterial community present in sorghum roots or rhisosphere (Mareque et al., 2018).

In the present study, we hypothesized that the natural levels of available nutrients, as well as the differences in soil pH, would impact the structural and compositional distribution of the native microbial communities. The main objective of this study was to compare bacterial community structure and function in the rhizosphere of sorghum cultivated areas that are impacted by cultivation practices using community-level physiological profile (CLPP) and 16S rRNA amplicon sequencing. The data obtained from this study provides an understanding of the bacterial community associated with this crop that could help to improve its sustainability.

2. Materials and methods

2.1. Site description and sampling

Rhizosphere soil samples were collected from five points/field-site of three different locations of sorghum cultivated areas in South Africa, and samples were designated as ASHSOIL1 ($26^0$57′29″S $27^0$24′07″E), ASHSOIL2 ($27^0$42′56″S $29^0$09′05″E), and ASHSOIL3 ($29^0$08′21″S $26^0$15′41″E). Normally sorghum is planted in South Africa from mid-October to mid-December. Sorghum is sensitive to low temperatures. The ideal soil temperature for germination is 15 °C at a depth of 10 cm. The crop is also sensitive to frost, and planting should be delayed until the last frost has passed. The rationale for selecting sampling sites was based on extensive sorghum cultivation i.e. chemically fertilized. The soils were clay loam in texture with a pH of 3.5 to 5.95. For microbial diversity analysis, multiple soil samples were collected from each location, with the subsamples being pooled to obtain a homogenous sample as per the method (Novello et al., 2017). Soil samples were stored on ice and immediately transported to the laboratory where they were stored at −20 °C for further use.

2.2. Soil rhizosphere chemical analysis

Bulk soil samples were collected from the sites to evaluate various soil characteristics, including C (%), P (mg/L), K (mg/L), Ca (mg/L), Mg (mg/L), Zn (mg/L), Exchangeable acidity (Exch. acidity) (cmol/L) and total cations (cmol/L) using standard protocols (Almodares and Hadi, 2009). Zinc (Zn) is one of the eight essential micronutrients. It is needed by plants in small amounts, but yet crucial to plant development. In plants, zinc is a key constituent of many enzymes and proteins.

2.3. Microbial community analysis using Biolog EcoPlates™

Community-level physiological profiles for each soil sample were assessed using the Biolog EcoPlate™ (BIOLOG Inc., CA, USA) assay. Each Biolog EcoPlate comprises wells containing 31 different types of carbon sources (10 different kinds of carbohydrates, 9 carboxylic or acetic acids, 6 amino acids, 4 polymers, and 2 amides/ amines) (Frąc et al., 2012). Analysis of each sample was conducted in triplicate. Estimation of CLPP for each soil sample was conducted as follows: 1 g of fresh soil was suspended in 10 ml sterile 0.85% NaCl solution and shaken vigorously at 150 rpm for about 60 min, then kept at 4 °C for 30 min to allow soil particles to settle (Siles et al., 2016). 150 μl of this soil suspension was added to each well, and the Ecoplates incubated at 27 °C for 5 days. The rate of substrate utilization is indicated by the reduction of tetrazolium dyes that are converted into colorless to purple. A well containing no carbon source was inoculated as a blank on each plate. The development of purple color in each well was measured as a change in optical density (OD). Absorbance at wavelength 595 nm was recorded every 24 h continuously for 5 days using an automated microplate plate reader (Synergy microplate readers (Bio Tek, US)). Microbial activity in each microplate expressed as an average well-color development (AWCD) was determined according to Eq. (1):

$$\mathrm{AWCD} = \frac{\sum ODi}{31} \quad (1)$$

Where richness (R) is the number of oxidized C substrates using an OD of 0.25 as the threshold value for a positive response (Garland, 1997). And *OD i* is the optical density value from each well after correcting by subtracting the OD value of the blank well and a total number of substrate were thirty one (Siles et al., 2016). The optical density of the eco plate at 72 h of incubation was used to calculate AWCD, and R, allowing for the best resolution.

2.4. 16S rRNA amplicon analysis

Total metagenomics DNA from each rhizosphere soil sample (0.25 g) was extracted using ZR Soil Microbe DNA MiniPrep (Zymo Research, Irvine, USA) by following the company protocols. The DNA was quantified using a NanoDrop™ micro-spectrophotometer and stored at −20 °C until further use. For pyrotagging, libraries of the V4 and V5 hypervariable regions of the bacterial 16S rRNA gene were amplified using E517F (5′-CAGCAGCCGCGGTAA-3′) and E969–984 (5′-GTAAGGTTCYTCGCGT-3′) primer pair (Wang et al., 2007; Wang & Qian, 2009). The whole sequencing process adapted from the protocol as mentioned on GS Junior 454 manufacturer's instructions.

Table 1
Soil characteristics of the rhizosphere soil collected from three sorghum fields.

Sample ID	C (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Exch. Acidity mol/l	Total cations cmol/l	pH	Zn (mg/L)
ASHSOIL1 (North West-South)	0.2	69	225	3660	283	0.07	21.24	5.93	3.8
ASHSOIL2 (Mpumalanga-West)	0.27	23	225	1562	678	1.92	15.87	3.5	3.2
ASHSOIL3 (Free State-NorthWest)	0.22	22	344	1202	325	0.17	9.72	4.59	1.9

2.5. Data analysis (Taxonomic distribution, statistical analysis, and functional analysis)

Roche 454 generated sequence reads were analysed through an open-source online server MG-RAST (Meyer et al., 2008) version 4.0.3 (http://metagenomics.anl.gov). The uploaded sequences were then quality trimmed using SolexaQA (Cox et al., 2010), to remove the low-quality regions and a k-mer based approach was used to remove the artificially duplicated reads. Annotations were made against the M5nr (Wilke et al., 2012) (an MD5 non-redundant database that utilizes several reference databases for sequence similarity search), with a minimum e value of 1E-5, identity cut off 60%, and a minimum alignment length of 15 bp. The comparative metagenomics studies were predicted by uploading the taxonomic abundance data obtained in biom-format from MG-RAST (Meyer et al., 2008) in METAGENassist (Arndt et al., 2012), which performs taxonomy to the phenotypic mapping of the datasets. Further data filtration and normalization were achieved through METAGENassist (Arndt et al., 2012). Unassigned variables and variables that remain near constant throughout the experiment were filtered through the interquartile range. Data normalization was carried out at default settings.

2.6. Diversity and statistical analysis

The adequacy of sampling was estimated through the rarefaction curve which was plotted based on the species count. A range of alpha diversity parameters was estimated using QIIME 1.9.1 (Caporaso and Kuczynski, 2010), Simpson's index was used to ascertain the uniform distribution of taxon within the soil samples. Shannon's index, which is a measure of microbial richness, was estimated along with Chao1 indices, PD whole tree, observed species, and Simpson's were calculated to determine the taxonomic diversification.

2.7. Core microbiome analysis

For core microbiome analyses, rarefied OTU tables were used for both soils for all the samples. QIIME command *compute_core_microbiome.py* was used to obtain a list of OTUs observed in all three samples.

2.8. Data availability

All the 16S rRNA amplicon sequences generated in the present study have been deposited at the MG-RAST server with the following accession numbers (ASHSOIL1: mgm4621557.3, ASHSOIL2: mgm4621558.3, ASHSOIL3: mgm4621559.3) and data are accessible by clicking on this link http://www.mg-rast.org/linkin.cgi?project=mgp12803. The data can also be made available on the request from the corresponding author, AK.

3. Results

3.1. Physicochemical properties of soil

Biochemical parameters of rhizosphere soil samples are summarized in Table 1, with the three samples showing observable differences. All three soil samples were acidic, however, ASHSOIL2 and ASHSOIL3 were more acidic than ASHSOIL1 with pH <5. Organic Carbon (%) was more or less the same in all the three soil samples, however, sample 2 (ASHSOIL2) had slightly more organic carbon (%) than sample 1 (ASHSOIL1) and 3 (ASHSOIL3). Total phosphorus level (mg/l) was low in soil samples 2 (ASHSOIL2) and 3 (ASHSOIL3) in comparison to ASHSOIL1. Total cations (cmol/L) in all soil samples were in the following order; (ASHSOIL1) > (ASHSOIL2) > (ASHSOIL3). The potassium (mg/L) was higher in the case of ASHSOIL3 (344 mg/L) compared to ASHSOIL1 and ASHSOIL2 (225 mg/L). The concentration of Ca (mg/L) was higher in ASHSOIL1 (3660 mg/L) followed by ASHSOIL2 (1562 mg/L) and ASHSOIL3 (1202 mg/L). However, the concentration of Mg (mg/L) was found to be higher in ASHSOIL2 (678 mg/L) followed by ASHSOIL3 (325 mg/L) and ASHSOIL1 (283 mg/L). The concentration of Zn (mg/L) was more or less the same for ASHSOIL1 (3.8 mg/L) and ASHSOIL2 (3.2 mg/L), but varied markedly in ASHSOIL1 (1.9 mg/L). Similarly the Exch. Acidity (mol/L) remained near-constant in ASHSOIL1 (0.07 mol/L) and ASHSOIL3 (0.17 mol/L) but changed significantly in ASHSOIL2 (1.92 mol/L).

3.2. Community level physiological profiling of rhizosphere samples

Community-level physiological profiles based on the ability of microorganisms to oxidize different carbon substrates, using microtiter plates with multiple sole-carbon sources, have become a popular tool for the comparison of microbial communities for their functionality and have been successfully used to differentiate microbial communities from several habitats such as soils (Gomez et al., 2004), water (Kaiser et al., 1998; Moll and Scott Summers, 1999; Jo et al., 2016) and solar salterns (Litchfield et al., 2001). The community structure and carbon metabolic activity of microbes have been shown as key indicators of soil quality. As an indicator of metabolic activity, AWCD of microbial communities in sorghum rhizosphere soil collected from three different field sites was ascertained using Biolog EcoPlate assays. ASHSOIL3 showed high AWCD values, followed by ASHSOIL1 and ASHSOIL2, respectively, which indicates the high metabolic activity of the microbial community, which increased with incubation time upto 5th days and gradually decreases after 6th-day of incubation (Fig. 1). However, metabolic activity among soil samples showed significant differences as shown in the figure. Similar substrate richness was indicated in two soil samples (ASHSOIL1 and ASHSOIL3) and comparatively less substrate richness was found in ASHSOIL2 (Fig. 2). Soil community structure or metabolic activity of the microbial community at different incubation times were estimated (Fig. 2).

3.3. Comparative bacterial community diversity analysis

The pyrosequencing of the 16S rRNA gene (V4-V5) from the bacterial communities present in samples from the three sites resulted in a total of 28,410 reads. The raw data obtained in fasta files were subjected to proper trimming and filtering. The 16S amplicon DNA sequencing of three different soil samples contained 3667 sequences totaling 1454,902 base pairs, 15,900 sequences totaling 6594,648 base pairs, and 8843 sequences totaling 34,84,142 base pairs for ASHSOIL1, ASHSOIL2, and ASHSOIL3, respectively. For ASHSOIL1, 294 sequences (8.0%) failed to pass the quality control (QC) pipeline. Of the sequences that passed QC, 2837 sequences (77.4%) contained ribosomal RNA genes. 536 (14.6%) of the sequences that passed QC had no rRNA genes. For ASHSOIL2, 142 sequences (7.2%) failed to pass the QC pipeline. Of the sequences that passed QC, 13,737 sequences (86.4%) contained ribosomal RNA genes. 1021 (6.4%) of the sequences that passed QC had no rRNA genes. For

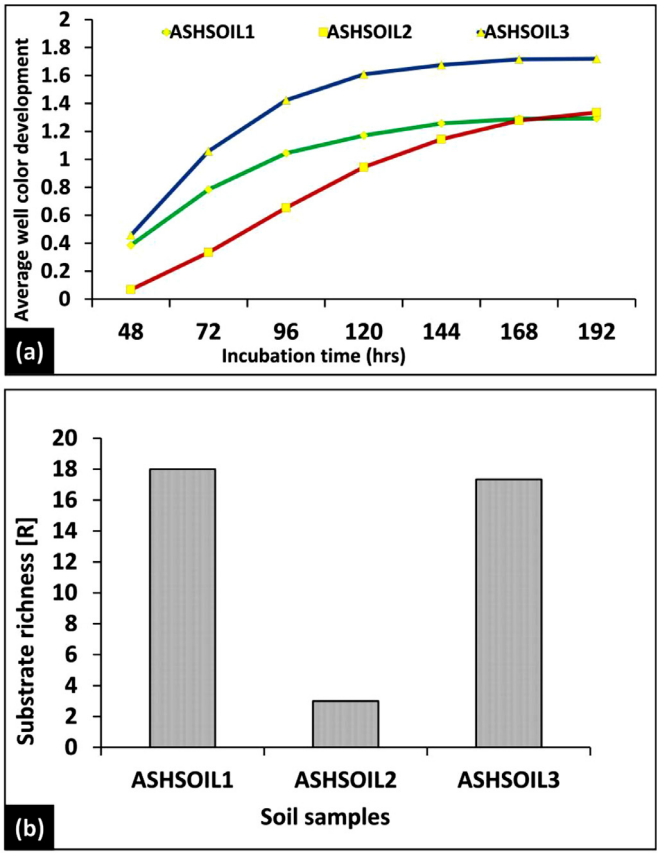

Fig. 1. **(a)** Comparative Average Well Color Development and **(b)** Comparative Substrate richness by bacterial communities present in three different sorghum rhizosphere soil samples (ASHSOIL1, ASHSOIL2 and ASHSOIL3).

ASHSOIL3, 744 sequences (8.4%) failed to pass the QC pipeline. Of the sequences that passed QC, 6444 sequences (72.9%) contained ribosomal RNA genes. 1655 (18.7%) of the sequences that passed QC had no rRNA genes. The taxonomy assignment was done using the MG-RAST pipeline (Meyer et al., 2008).

3.3.1. Domain-level classification of microbial communities in sorghum rhizosphere

The domain level distribution of the sample ASHSOIL1 was Bacteria (96.5%) and Eukaryota (3.5%), while ASHSOIL2 contained Bacteria (99.6%) with Eukaryota (0.4%) and ASHSOIL3 composition was Bacteria (95.4%) with Eukaryota (4.6%).

3.3.2. Phylum-level classification of microbial communities in sorghum rhizosphere

Representations of the phylum level microbial diversity across the three different sites for the top 6 taxa are given in (Fig. 3). The major percentage of reads was assigned to Cyanobacteria, which dominated the bacterial community at the phylum level with a relative abundance of 81.6% for ASHSOIL2, 71.8% for ASHSOIL3 followed by 63.3% for ASHSOIL1.

The relative abundance of Bacteroidetes was found to be higher in ASHSOIL1 (23.5%) followed by ASHSOIL3 (19.5%) and ASHSOIL2 (11.1%). The relative abundance of phylum Proteobacteria was higher in ASHSOIL1 (9.0%) followed by ASHSOIL2 (6.0%) and ASHSOIL3 (3.7%). Actinobacteria were identified in all three samples with Chlorophyta, significantly, being higher in abundance in ASHSOIL3 and ASHSOIL1 (Fig. 3) as compared to ASHSOIL2, where Actinobacteria is slightly higher in percentage than Chlorophyta (Fig. 3).

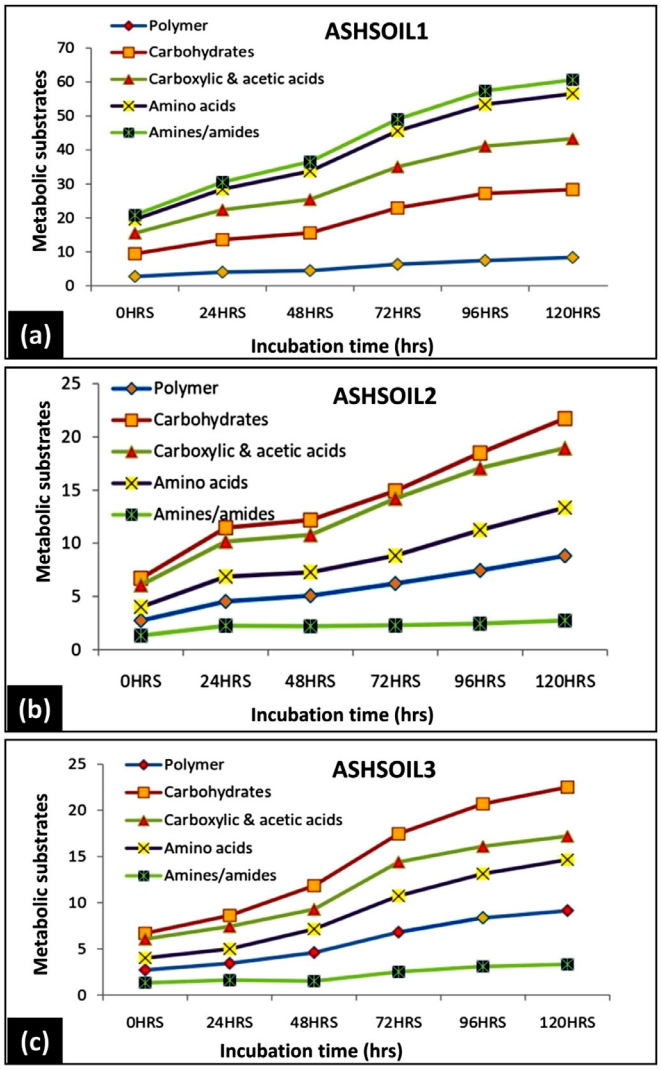

Fig. 2. Metabolic activity of microbial community of different soil samples; (a) ASHSOIL1,(b) ASHSOIL2,(c) ASHSOIL3 at progressive incubation times (hrs).

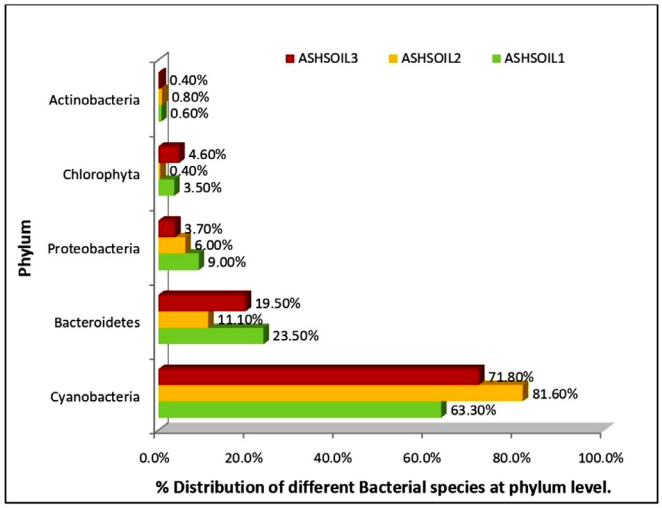

Fig. 3. Phylum-level classification and relative abundance of microbial communities in sorghum rhizosphere.

Table 2
Alpha and Beta diversity ((a) unweighted and (b) weighted) of the three different samples.

Alpha Diversity Samples	Chao1	Shannon	PD_whole_tree	Simpson	Observed species
ASHSOIL1	1587.889	6.453711	12.60169	0.83	792
ASHSOIL2	3017.375	4.759217	14.04041	0.70	1520
ASHSOIL3	3668.89	6.08862	11.94011	0.76	1662

	ASHSOIL1	ASHSOIL2	ASHSOIL3
a) Unweighted			
ASHSOIL1	0.0	0.432	0.450
ASHSOIL2	0.432	0.0	0.404
ASHSOIL3	0.450	0.404	0.0
b) Weighted			
ASHSOIL1	0.0	0.1302	0.0858
ASHSOIL2	0.1302	0.0	0.0791
ASHSOIL3	0.0858	0.0791	0.0

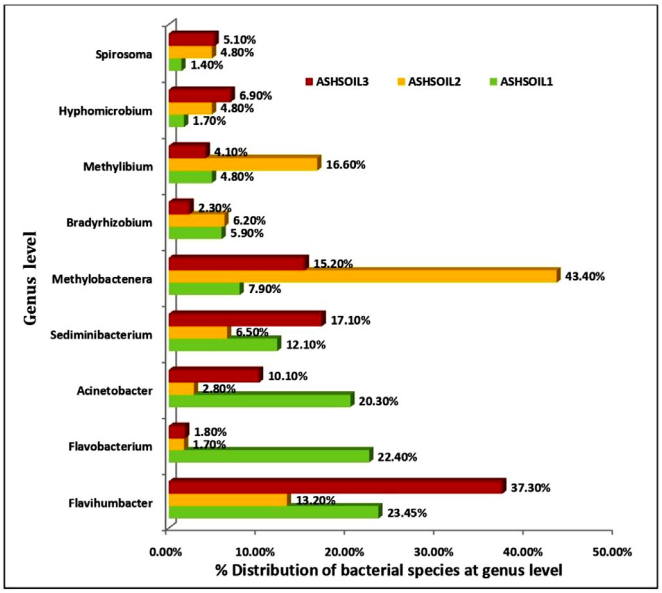

Fig. 4. Taxonomic classification and relative abundance of microbial communities in sorghum rhizosphere at the genus level.

3.3.3. Taxonomic classification of microbial communities in sorghum rhizosphere at the genus level

Genus level taxonomic distribution of the top 9 most abundant microbial representatives shows that genus *Flavihumibacter* dominated the composition with relative abundance ranging from 13 to 3% across all the sites (Fig. 4). *Methylotenera* was found in large numbers across all the samples with a notable decrease in relative abundance from ASHSOIL-2 (43.4%) to ASHSOIL-3 (15.2%) and ASHSOIL-1 (7.9%) (Fig. 4).

The relative abundance of the *Acinetobacter* and *Hyphomicrobium* ranged from 2 to 0.3% and 1.7- 6.9%, respectively, across the samples (Fig. 5). The relative abundance of the rest of the genera, such as *Flavobacterium, Sediminibacterium, Bradyrhizobium, Methylibium,* and *Spirosoma*, is shown in Fig. 4. Microbial composition in the three sorghum rhizosphere samples is shown as (a) heat map of the top 11 microbiomes at phyla level and (b) top 26 taxa at the genus level (Fig. 5).

3.3.4. Analysis of species diversity

The diversity matrices were generated by considering the median values. The microbial diversity of the three sorghum rhizosphere samples was estimated by calculating the alpha diversity metrics of species richness and evenness. The alpha diversity indices (Chao1, Shannon, Observed Species, Simpson's, and phylogenetic diversity (PD whole tree)) were used to estimate the diversity within each sample. Chao1 metrics are commonly used as species richness estimator within a sample and is based on the number of rare operational taxonomic units, or OTUs, present within a sample (Table 2). Overall, ASHSOIL3 (3668.89) showed the highest richness, followed by ASHSOIL2 (3017.375) and ASHSOIL1 (1587.889), and the species richness increased with an increase in the number of sequences for all the samples. In the case of the Shannon index, the bacterial diversity was found to be highest at 6.453 in ASHSOIL1, followed by 6.088 in ASHSOIL2 and 4.759 in ASHSOIL3 (Table 2).

The diversity of the ASHSOIL3 was lower compared to ASHSOIL1. Simpson's diversity index is used to estimate both the number and the relative abundance of each species. Simpson's diversity was found to be highest in ASHSOIL1 (0.833) followed by ASHSOIL3 (0.764) and ASHSOIL2 (0.700) (Table 2). Total Observed species and PD_whole_tree were found to be highest in ASHSOIL3 (1662), followed by ASHSOIL2 (1520) and ASHSOIL1 (792), and ASHSOIL2 (14.040), ASHSOIL1 (12.6016), and ASHSOIL3 (11.940), respectively (Table 2). Beta diversity was used to estimate microbial diversity across the three soil samples. The phylogenetic similarity of the samples was measured using weighted and unweighted unifrac distances. Inter sample variations were observed with ASHSOIL1 (0.43), ASHSOIL2 (0.40) and ASHSOIL3 (0.45). Similarly, in terms of weighted unifrac distances variations were observed between the samples with ASHSOIL1 having (0.13), ASHSOIL2 (0.0791), and ASHSOIL3 (0.08) phylogenetic distance (Table 2).

3.4. Core microbiome analysis

Core microbiome analysis was performed to clarify the presence/absence of keystone taxa. Out of 3412 clustered OTUs, we found 95 OTUs that were consistently present in the rhizosphere of all three *Sorghum* samples grown in three different geographically distinct areas. All, these OTUs (146), classified up to the genus level, represented only 4.279% of the total OTUs (3412), but 78.825% of all sequences (28,402). This core Sorghum rhizosphere microbiome consisted of 95 OTUs, with Proteobacteria (46) as the most abundant contributor with the mean relative abundance of 48.42%, followed by Bacteroidetes (21 OTUs, 22.10%), Actinobacteria (7 OTUs, 7.36%), Cyanobacteria (7 OTUs, 7.36%), Planctomycetes (6 OTUs, 6.31%), Verrucomicrobiae (2 OTUs, 2.10%), Chlamydiae (2 OTUs, 2.10%), Armatimonadetes (2 OTUs, 2.10%), TM7 (1 OTUs, 1.04%), Chloroflexi (1 OTUs, 1.04%) (Fig. 6).

3.5. Taxonomic to the phenotypic mapping of sorghum rhizosphere metagenome

Extensive analysis of metagenomic data by METAGENassist (Arndt et al., 2012) revealed significant differences in the metabolic composition of the three (ASHSOIL1, ASHSOIL2, and ASHSOIL3) metagenome samples (Fig. 7). The representations of the abundance of the function from the three soil samples revealed the significance

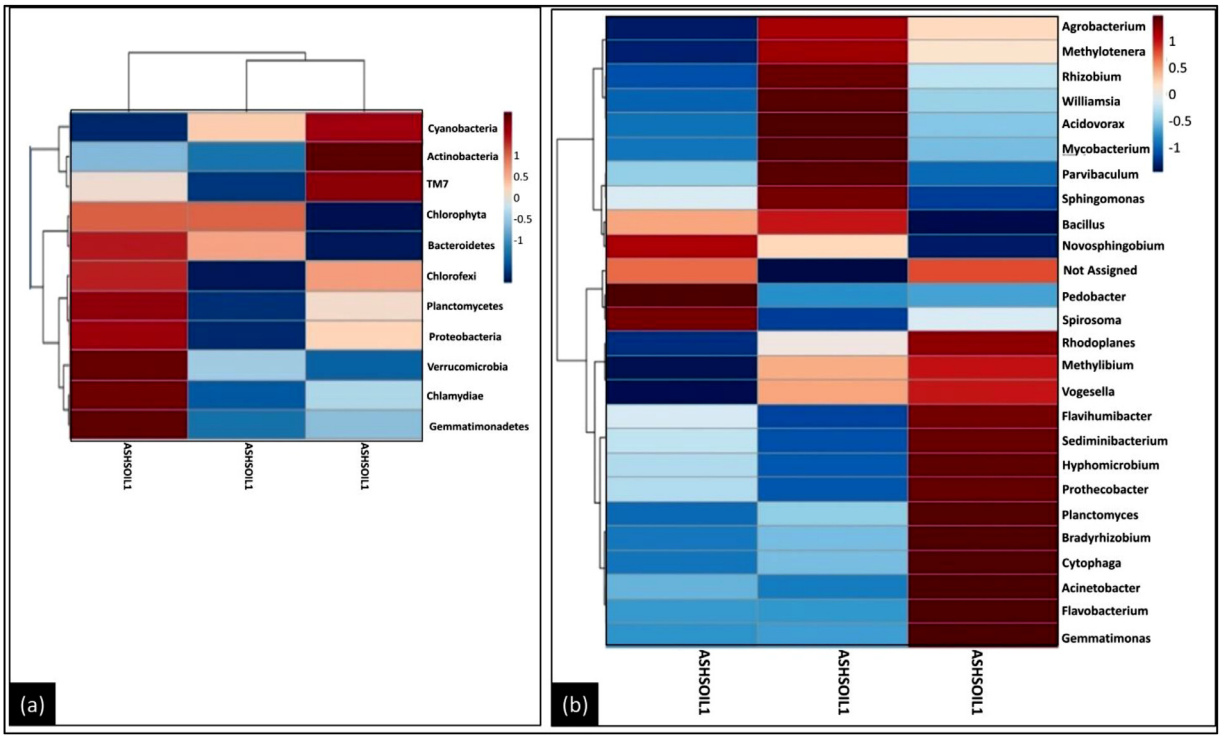

Fig. 5. Microbial composition in the three sorghum rhizosphere samples. (a) heat map of the top 11 microbiomes at phyla level and (b) top 26 taxa at the genus level.

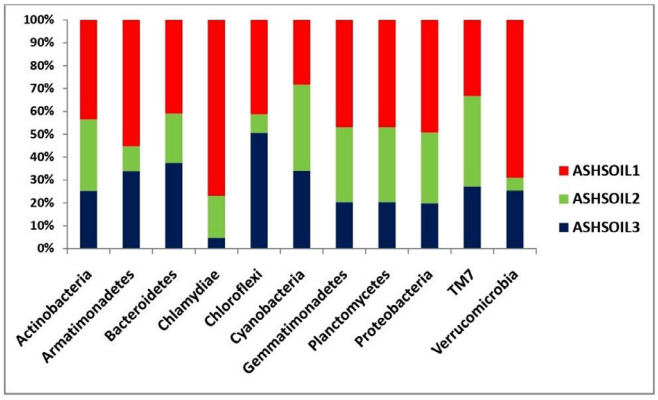

Fig. 6. Core microbiome of the sorghum rhizosphere representing the relative abundance of bacteria at the phylum level.

of certain processes, including nitrite reduction, dehalogenation, sulfate reduction, ammonia oxidation, and sulfide oxidation. The relative abundance of dehalogenating bacteria was found to be high in all three samples with a relative abundance of 22.1%, 12.2%, and 17.8% corresponding to three soil samples ASHSOIL1, ASHSOIL2, and ASHSOIL3, respectively.

ASHSOIL1 showed a high relative abundance of ammonia oxidation (21.1%) and sulfide oxidizers (18.3%); whereas in ASHSOIL2, only 11.1% of microbiota belonged to ammonia oxidizers and 10.8% were sulfide oxidizers. Similarly, ASHSOIL3 consisted of 17.3% ammonium oxidizers and 16.5% sulfide oxidizers. All three samples were also found to comprise sulfate-reducing bacteria, with the percentage abundance ranging from 5.3%, 3.0%, and 2.1%, respectively, for ASHSOIL1, ASHSOIL2, and ASHSOIL3. Nitrite reducers were also found to be present in all three samples, with ASHSOIL1 having a high abundance of 5.0%, compared to ASHSOIL2 with 2.6% and ASHSOIL3 with 2.0% the data can be viewed by following this link http://www.metagenassist.ca/METAGENassist/faces/Secure/Analysis/AnalysisView.jsp?form1:NavigationBar:naviTree:download:download_link_submittedLink=form1:NavigationBar:naviTree:download:download_link.

4. Discussion

In this study, we profiled the microbial communities from the rhizospheres of sorghum, grown under different agricultural management practices in three different geographical areas. The bacterial communities were analyzed using culture-independent 16S rRNA amplicon sequencing. To the best of our knowledge, we are the first to explore CLPP and16S rRNA amplicon sequencing of the bacterial community in a sorghum rhizosphere. Here, in this study, we showed how the management practices and edaphic factors shape microbial diversity. From the present study, the species abundance analysis revealed the effect of management practices and selection pressure. Various management practices (application of fertilizers, organic matter) are found to be the probable drivers of the differences in species abundance distributions, whereas the abiotic factors particularly pH are the most probable selection pressures that shape the bacterial diversity among the three samples. Microbial diversity is a function of pH, with low pH (acidic) soils having lower diversity. Plants have been found to shape the specific bacterial communities colonizing their rhizospheres and these are very important for improving plant growth (Sugiyama et al., 2014), large numbers of bacterial populations have been reported to bloom in the rhizosphere due to the attraction created by root exudates and the microenvironment provided by nearby plants (Mendes et al., 2013; Chaparro et al., 2014; Huang et al., 2014; Saleem et al., 2018), along with abiotic factors in the soil. Sugiyama et al. (2014) used similar techniques, including metagenomics and CLPP, to explore the shift in the bacterial community present in the rhizosphere under different developmental stages of the soybean plant. A similar study was conducted by Colin et al. (2017), to understand the taxonomic and functional differences in the beech rhizosphere microbiome across the natural soil topo sequences. Wu et al. profiled the microbial community structure of the soil and the metabolic activity of Pinus elliottii plantations across different stand ages in a subtropical area (Wu et al., 2015) using CLPP and phospholipid fatty acid analysis. They found that soil nutrient and C/N ratio contributed most

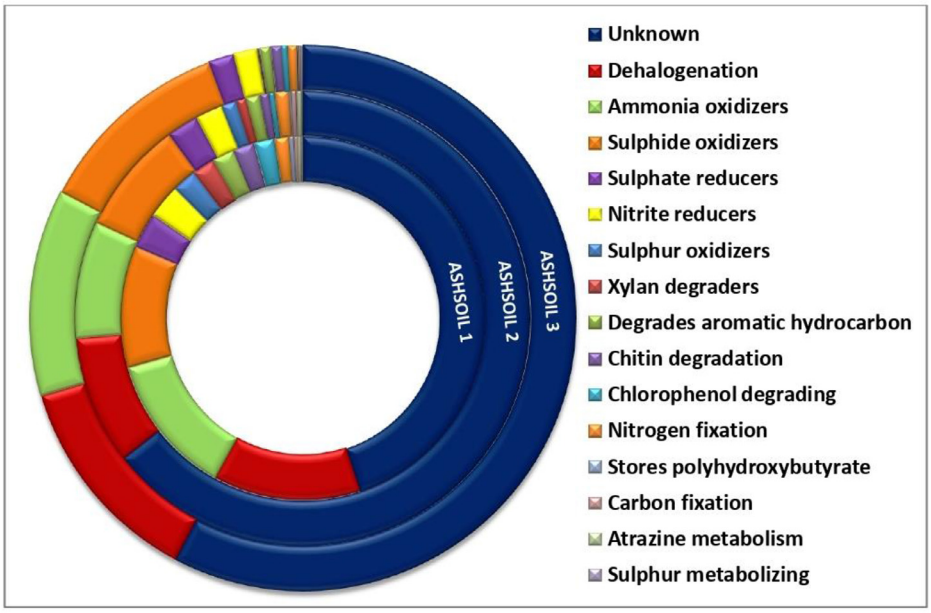

Fig. 7. Putative metabolic requirements of the three different sorghum rhizosphere metagenome samples.

Metabolic functions	ASHSOIL1	ASHSOIL2	ASHSOIL3
Unknown	75.60%	86.20%	81.30%
Dehalogenation	22.10%	12.20%	17.80%
Ammonia oxidizers	21.10%	11.10%	17.30%
Sulphide oxidizers	18.30%	10.80%	16.50%
Sulphate reducers	5.30%	3.00%	2.10%
Nitrite reducers	5.00%	2.60%	2.00%
Sulphur oxidizers	3.60%	1.40%	0.10%
Xylan degraders	3.40%	1.00%	0.10%
Degrades aromatic hydrocarbon	3.40%	1.30%	0.90%
Chitin degradation	3.20%	0.90%	0.90%
Chlorophenol degrading	3.00%	0.50%	0.50%
Nitrogen fixation	2.20%	1.40%	0.80%
Stores polyhydroxybutyrate	0.70%	0.20%	0.08%
Carbon fixation	0.60%	0.50%	0.10%
Atrazine metabolism	0.60%	0.50%	0.10%
Sulphur metabolizing	0.20%	0.10%	0.20%

significantly to microbial community structure and metabolic activity in different stand ages of *P. elliottii* plantations. Using Biolog EcoPlate microtitre substrate utilization assays, we found that the metabolic capabilities of microorganisms in sorghum rhizosphere soil ASHSOIL1 and ASHSOIL3 were higher when compared to that of ASHSOIL2 (Fig. 1). Interestingly, carbohydrate usage was highest in ASHSOIL2 and ASHSOIL3, with the use of amides and amines relatively low, while in ASHSOIL1 use of amides and amines was highest, and carbohydrate usage lower. These results indicate differences in bacterial community structure between the three sorghum rhizospheres and maybe a reflection of different soil environments, as soil analysis indicated that in ASHSOIL1 P and Ca levels, as well as pH, were higher than in the other two samples. An underlying mechanism of this shift in the rhizosphere microbial diversity may be due to the harsh abiotic conditions (low pH) in these soils, which may have affected the quality and quantity of root exudates released into the surrounding soil. The soil profile is one of the key determining factors of microbial diversity (Hansel et al., 2008). Land use patterns, along with agricultural practices, alter the microbial communities by altering soil properties, thus affecting soil microbial diversity and function (Gong et al., 2009; Ai et al., 2012). There is some understanding of how soil pH and nutrient availability interact to shape the microbial community composition (Geisseler and Scow, 2014), and our results highlighted differences in the microbial composition among the three sites investigated. Quantification of soil microbial communities is vital for understanding and exploring many aspects of soil microbial ecology (Dubey et al., 2019b; Malla et al., 2019). The largest numbers of Operational Taxonomic Units (OTUs) that correlated with the soil physicochemical profiles were positively correlated with the soil pH and soil nutrient content; however, OTUs affiliated to Cyanobacteria were found to be more abundant in soils having low pH. The dominance of this phylum (Cyanobacteria) is possibly because of nutrient richness in these soils; similar results indicating the dominance of Cyanobacteria were reported by Zhang et al. (2018). In their study Zhang et al. (2018) examined changes in algal and cyanobacterial communities, nutrients, carbon, and composition of dissolved organic matter in topsoil, and the interaction among the community and soil type, using redundancy analysis. The relative abundance of Bacteroidetes is comparatively higher in ASHSOIL1 and ASHSOIL3 compared to ASHSOIL2 (Fig 3) and with the pH of ASHSOIL1 (5.93) and ASHSOIL3 (4.59) being higher side than ASHSOIL2 (3.5) (Table 1), this positive correlation/ trend suggests that the microorganisms within the Bacteroidetes group are positively correlated with soil properties. Indeed, our results are strongly in agreement with the findings of Curd et al. (2018), wherein they characterized heterogeneity in soil, as well as alpha- and beta-diversity of bacterial communities using 16S rRNA gene sequencing. They concluded that bacterial diversity is positively correlated with heterogeneity in the soil. In this study, these sites in South Africa have been selected on the basis of extensive sorghum cultivation and the applications of chemi-

cal fertilizers for improving the growth and yield. But how this long term cultivation practices affect the bacterial community, distribution and functional potential is a matter of investigation. In our study, we found a lower relative abundance of Actinobacteria across all the samples, and this is possibly because of lower pH values and CPK ratio. Similar results were shown by Wang et al. (2017). They found that the microbial community colonizing soil changes at developmental stages of the plant due to changes in the composition of root exudates and pH of the soil. The slightly increased relative abundance of Proteobacteria and Chlorophyta from the more acidic to less acidic soil in our study strongly agrees with the results of Zhang et al. (2017). Zhang et al. (2017) used 454 pyrosequencing, targeting the V1-V3 variable regions of 16S rRNA genes, to explore bacterial diversity and community structure present in the soil after 7years of fertilization. Results of their study add support to our study, as the relative abundance (%) of most bacterial phyla was higher in near-neutral than in acidic or alkaline soils. The most dominant were Proteobacteria, followed by Actinobacteria. Their study concluded that after 7 years of applying NPK fertilizers, community structure and bacterial diversity present in the soil were more shaped by changes in the pH of the soil than by the direct effect of adding nutrients. In addition, a study conducted by Tian et al. (2018) found that bacterial community also changes with change in geographical area, supporting our findings that the dissimilarity in bacterial communities can also be attributed to different geographic regions, and environmental variables, such as climatic factors. The members of core microbiome shared by all the three samples were in general very abundant, and include the taxa from Cyanobacteria, Bacteroidetes, Proteobacteria, Actinobacteria, Armatimonadetes, Chloroflexi, Chlamydiae, Saccharibacteria (TM7), Verrucomicrobia, and Planctomycetes. Alpha diversity metrics of the sorghum rhizosphere microbiome showed stochastic variations between the three sampled sites. Beta diversity analyses measured by weighted and unweighted UniFrac distance matrices showed that the samples were structurally and phylogenetically distinct. Taxonomic to phenotypic mapping results further confirmed the differences between the three sorghum rhizosphere communities profiled. Aside from the three most abundant phenotypes, present in all three samples (ammonia oxidizer, dehalogenation, and sulfide oxidizer), and relative abundances of many other phenotypes were larger in ASHSOIL1 than in the other 2 samples, notably: chitin, chlorophenol, aromatic hydrocarbon and xylan degradation, nitrite and sulfate reduction, sulfur oxidation and nitrogen fixation. All of these phenotypic capabilities amongst the soil-dwelling microbes would be of value to the plants occupying the same rhizosphere, contributing to nutrient availability and pollution remediation, and ultimately to a potential increase in crop production.

5. Conclusions

For an accurate appraisal of a particular microbial ecosystem, it is necessary to integrate the influences of biotic and abiotic factors on the community structure and biodiversity. Based on the community level physiological profiling and the metagenomics and subsequent *Insilico* data analysis, the three sorghum cultivated sites from different geographic locations, shown in this study were found to be colonized by diverse microbial communities. Differences in the soil bacterial communities were likely related to differences in soil properties arising from geographic location and/or agricultural management practices, such as intensive agrochemical applications. Differences in community structure among the three sites correlated with variation in soil pH, as well as soil nutrient content. The contribution of microbial communities to the enhancement of nutrient acquisition by plants is well documented; therefore knowledge of the community structure and the metabolic capabilities of its members, as highlighted in our results, could be invaluable in predicting the success of a particular agricultural crop. Clear details on specific abiotic factors, as well as agricultural management practices should be considered when exploring microbial communities associated with specific plants, particularly in respect of crops. Further investigation into potential differences in the growth of sorghum, in response to the community composition as revealed in this study, is recommended. Keeping in view the future needs, further experimentation is required to decipher the impact of these enriched microbes on the growth, maintenance, and health of the Sorghum plant, moreover, it is also important to mention that core microbiome analysis discussed here in the present study is exclusively based on taxonomy and that functional the traits need to be taken into account for better and more elaborative insight into the impact of habitat expansion and management practices on the microbiome.

Ethics declarations

The whole study and experimental analysis were conducted by Dr. Kumar during his Postdoctoral Fellowship at Rhodes University, Grahamstown South Africa under the mentorship of Prof. J. Dames.

Declaration of Competing Interest

The authors declare no competing interests.

CRediT authorship contribution statement

Ashwani Kumar: Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Anamika Dubey:** Visualization. **Muneer Ahmad Malla:** Visualization. **Joanna Dames:** Supervision.

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